‘tesA PCR Protocol:

(a). Using TaKaRa Ex Taq™ kit

1. Prepare 50 µl reaction in 0.5 ml PCR tube on ice

	Volume	Final concentration
TaKaRa Ex Taq (5 units/µl)	0.25µl	0.025 units
10X Ex Taq Buffer	5µl	1X
dNTP Mixture (2.5 mM each)	4µl	200µM
DNA template	Depend on concentration	±300ng
Forward Primer (10x)	5µl	1X
Reverse Primer (10x)	5µl	1X
Sterilized distilled water	Up to 50µl	

2. Mix gently by vortex and briefly centrifuge to collect all components to the bottom of the tube.
3. Transfer PCR tubes from ice to a PCR machine and then run with the following PCR profile

	Temperature	Time
Denaturation	98℃	3 minutes
10 cycles	98℃	30 seconds
	57℃	30 seconds
	72℃	1 minute
20 cycles	98℃	30 seconds
	64℃	30 seconds
	72℃	1 minute
Final extension	72℃	10 minutes
Storage	12℃	∞

(b). Using TaKaRa LA Taq™ kit

1. Prepare 20 µl reaction in 0.5 ml PCR tube on ice

	Volume	Final concentration
TaKaRa Ex Taq (5 units/µl)	0.2µl	0.05 units
10X Ex Taq Buffer	2µl	1X
dNTP Mixture (2.5 mM each)	4µl	200µM
DNA template	Depend on concentration	±300ng
Forward Primer (10x)	1µl	0.5X
Reverse Primer (10x)	1µl	0.5X
Sterilized distilled water	Up to 20µl	

2. Mix gently by vortex and briefly centrifuge to collect all components to the bottom of the tube.
3. Transfer PCR tubes from ice to a PCR machine and then run with the following PCR profile

	Temperature	Time
Denaturation	98℃	3 minutes
10 cycles	98℃	30 seconds
	57℃	30 seconds
	72℃	1 minute
20 cycles	98℃	30 seconds
	64℃	30 seconds
	72℃	1 minute
Final extension	72℃	10 minutes
Storage	12℃	∞